Application of Elemental Fingerprinting to Evaluate the Dynamics of Larval Exchange

Lisa A. Levin
Integrative Oceanography Division
Scripps Institution of Oceanography
La Jolla, California 92093-0218
phone: (858-534-3579) fax: (858-822-0562) email: llevin@ucsd.edu

John Largier
Integrative Oceanography Division
Scripps Institution of Oceanography
La Jolla, California 92093-0218
phone: 858-534-6268 fax: 858-534-0300 email: jlargier@ucsd.edu

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http://levin.ucsd.edu/

LONG-TERM GOALS

The primary goal of this research is the development of trace elemental fingerprinting methods for the assessment of larval exchange among bivalve populations. This methodology will employ larval and recruit origin determinations using shell microchemistry in mytilid mussels. Comparisons of realized larval exchange with that predicted by currents can provide insight about controls on population connectivity. The ultimate goal is to incorporate dispersal information into population dynamic models to better understand the consequences of different patterns of population connectivity.

OBJECTIVES

The objectives of this research are to: (1) develop trace elemental fingerprinting methods for mytilid mussels in southern California and (2) determine the extent and conditions under which mussel populations are self seeding or receive external recruits. This year's work focuses on validation of fingerprinting methods, verifying that site-specific trace element signatures are imparted to the larval and recruit shell and that these signals are stable in time. To this end we have adopted new laser ablation methods in place of solution-based analyses. Following validation work, specific ecological questions addressed are the extent to which bay and coastal populations are self-seeding, the importance of distance or circulation in determining population connectivity, and the variability in recruit sources over time.

APPROACH

Elemental "fingerprinting" utilizes a naturally induced tag that incorporates specific environmental signals such as trace elements, salinity or temperature, to track movements of larvae. We have adopted 3 means to validate the method: (1) analysis of shell composition for larvae spawned in the lab and out-planted at known sites (2) characterization of the composition of shell material freshly deposited by new recruits and (3) quantification of seawater composition at various locations. For the method to work, the seawater at each site must impart distinctive elemental signatures to the shell. Because bivalves retain the larval shell after settlement, it is possible to obtain a time record of composition that indicates probable larval trajectories and sites of development.

For this study, we collected newly settled, post-larval recruits in San Diego Bay, neighboring embayments, and surrounding nearshore coastal habitats. Molecular PCR techniques were developed to distinguish newly settled *Mytilus californianus* from *M. galloprovincialis* using soft tissues. The elemental composition of larval and post settlement shell in recruits < 1 mm was determined with an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) either in solution or via laser ablation. Discriminant Function Analyses (DFA) were used to determine canonical variables (linear combinations of sampled trace elements) that serve as 'fingerprints' to identify the locations where larvae developed.

WORK COMPLETED

This year's research efforts involved: (1) Establishment of mussel culturing and field out-planting techniques. We have successfully reared *M. californianus* from spawn until settlement and out-planted both this species and *M. galloprovincialis*, raising them in situ for 1 week in PVC 'larval homes'. Solution- and laser-based analyses of these shells are underway. (2). Development of laser ablation methods to analyze shell composition. Methods for cleaning and mounting shells, instrument settings such as laser intensity, form (spot/raster) and duration as well as computer data collection techniques have been tested. (3) Creation of primers that distinguish DNA in *M. californianus* from that of *M. galloprovincialis*. (4) Weekly sample collection of new recruits and surface water at SIO Pier and in San Diego Bay to assess temporal variation in trace element signatures. (4) Quarterly sample collection at 14 bay and coastal sites in southern California. (5) Continued work on methods for analysis of seawater elemental concentrations and removal of matrix effects. (6) Characterization of temperature regimes (with thermistor records) at bay and coastal sites to interpret temperature-sensitive elements (e.g., Sr, Mg, Ba) and to assess environmental variability at the scale of the dispersal process.

RESULTS

Spatial variation.

Laser ablation analysis confirmed among-site differences necessary to implement elemental fingerprinting studies of mussel dispersal. New *M. californianus* recruits (<2.5mm, average 1.3 mm) collected during a two day period in December 2001 were analyzed for trace element composition using LA-ICP-MS by ablating a transect of three lines corresponding to late settler, early settler, and larval shell, respectively. A DFAs conducted with SYSTAT software on ten isotopes (Mg_{24}, Cr_{52}, Cu_{63}, Zn_{64}, Zn_{66}, Sr_{88}, Ba_{138}, Sn_{118}, Pb_{206}, U_{238}) indicated our ability to distinguish late settlers among the sites with 95% accuracy (Fig. 1A). Sr, Zn, Mg, and U were the most useful elements. Open coast sites, when considered alone, could be distinguished with 100% accuracy, mostly due to the concentrations of Pb, Zn, and Cr.

Previous analyses of the whole shell using solution-based methods indicated that shell size had a significant effect on its elemental fingerprint. The laser ablation-based methods allowed us to target specific parts of the shell, and we have found there is no relationship between total size of an individual (< 2 mm) and the elemental signal in its shell. However, the different sections of the shell did exhibit significant differences in signal, regardless of the origin of the individual. A discriminant rule distinguished the larval shell from the settler shell 92% of the time, mostly due to Mg and U. The different parts of the settler were also distinguishable, mostly due to Sr. When the Mg/Sr ratio of the larval shell, early settler, and late settler are compared, all were significantly different. This result probably reflects the changing ratio of aragonite to calcite as the bivalve shell grows. This fact accentuates the need to compare similar ontogenetic stages when assigning larvae to origins using trace elements.

Temporal Variation.

Trace elemental composition of new recruit shell edge at a single site during 4 weeks in Feb. 2002 (SIO Pier) exhibited significant temporal variation. We noted instability in elemental composition for Cr ($P=0.006$), Ag ($P=0.021$), and Cd ($P=0.022$) (Kruskall Wallace test), and no significant change for Pb, Mg and Mn. Recruitment variation, measured as settling mussels per g byssus thread, was noted among sampling dates. The sampling date with 3-4x higher recruitment than the other weeks yielded settlers with lower Cr, Ag, Mg and Cd concentrations, perhaps indicating input of oceanic or upwelled water (Fig. 1B). Multiple analyses of the same shells on different days revealed no machine drift, analysis date or firing error effects for Mg, Mn, Cr, Ag, and Pb ($P>0.05$ Wilcoxon signed rank test). For elemental fingerprinting to aid interpretation of dispersal, either temporal variation within each site must be lower than that among sites, or sites must be sampled during the period when larvae are developing (ideally both should occur). The comparison of temporal and spatial variation of signatures in the southern California Bight is a high priority and is required for full validation of the fingerprinting method.

IMPACT/APPLICATIONS

This research will advance understanding of marine invertebrate dynamics by (a) adapting laser ablation techniques to the study of bivalve larval origins and exchange, (b) relating physical exchange probabilities to actual estimates of bay-ocean and bay-bay larval exchange, and (c) providing practical application of connectivity information to the management of mussel populations in protected areas (e.g., Cabrillo Nat'l Monument). Expansion of element-based tagging approaches to identification of invertebrate recruit origins, and to questions of populations connectivity should open up a wide range of applications including assessment of the interdependence of different habitats, evaluation of spatial and temporal variability in recruit dynamics, and determination of pollution consequences.

TRANSITIONS

During the award period we have made a technological transition from ICP-MS solution-based analysis to LA-ICP-MS solid phase analyses, opening an enormous opportunity for the study of invertebrate shells as recorders of their environmental history.

RELATED PROJECTS

We are working with J. Gieskes and C. Mahn (Scripps) who are examining trace metal concentrations in waters and sediments of San Diego Bay. They are assisting with methods development in analysis of seawater elemental composition. We are collaborating with participants in a UC Marine Council California Environmental Quality Initiative that aims to develop a trace elemental atlas for dispersal studies in California. Funded through this program are Scripps graduate student Bonnie Becker, who is studying the influence of the Point Loma kelp forest on scales of dispersal and recruitment in *M. californianus* at the Cabrillo National Monument, San Diego and graduate student Joel Fodrie, who is using trace element fingerprinting to study utilization of estuaries by juvenile halibut. Our thermistor data and transport patterns described along the open coast complement similar efforts to synthesize historical hydrographic data for the region in other projects.

PRESENTATIONS

Lisa Levin, Bonnie Becker, Pat McMillan, Joel Fodrie, John Largier, Shelly Walther
Integrative Oceanography Division, Scripps Inst. Oceanography
Claudio Di Bacco, Biology Dept. Woods Hole Oceanographic Inst
Use of Elemental Fingerprinting to Evaluate the Dynamics of Larval Exchange.
Presented at: Western Society of Naturalist 2001 Ventura, CA
ASLO/AGU Ocean Sciences 2002 Honolulu, Hawaii
Benthic Ecology Meeting 2002 Tallahassee, Fla

Lisa Levin, Bonnie Becker, Pat McMillan, Joel Fodrie, John Largier, Shelly Walther
Integrative Oceanography Division, Scripps Inst. Oceanography
Claudio Di Bacco, Biology Dept. Woods Hole Oceanographic Inst
Construction of Larval Trajectories Using Trace Element Concentrations in Mytilid Larval Shells.
ASLO 2002 Victoria, Canada

Bonnie Becker, Lisa Levin, Pat McMillan, Joel Fodrie, Shelly Walther
Integrative Oceanography Division, Scripps Inst. Oceanography
Use of Trace Element Concentrations in Mytilid Mussel Shells to Determine Larval Sources. Larval Biology Meeting 2002 Vigo, Spain

Bonnie Becker, Lisa Levin, Pat McMillan, Joel Fodrie, Shelly Walther
Integrative Oceanography Division, Scripps Inst. Oceanography
Mussels, Lasers, and Marine Reserves: Why we care about the chemistry of baby mussel shells. North County Sierra Club, San Diego, CA; September 2002

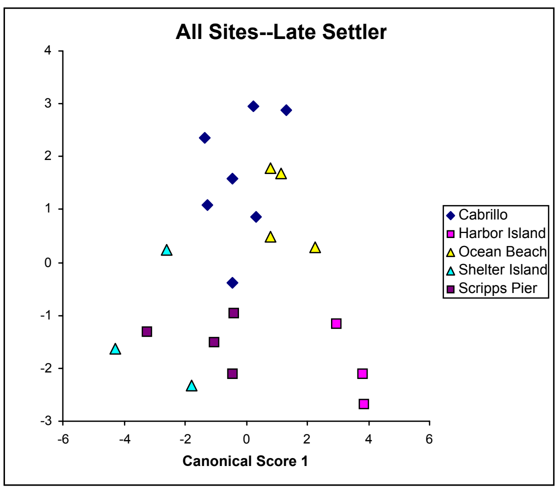

Fig. 1A. Spatial Variation. Discriminant plot of trace element composition for newly recruited mussels from 3 open coast and 2 San Diego Bay sites (Harbor and Shelter Island) in southern California.

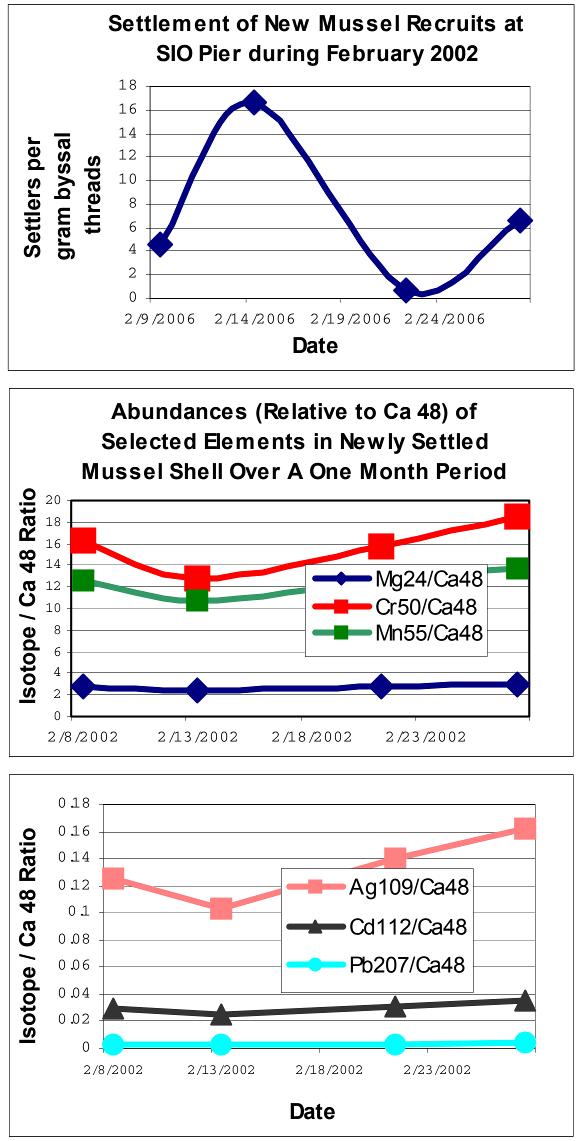

Fig. 1B Temporal Variation. Weekly variation in recruitment and shell edge elemental composition during February 2002.